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Who should be screened for chromosomal abnormalities before ICSI treatment?

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ABSTRACT: Guidelines on karyotyping infertile men before ICSI treatment are not consistent. Most guidelines recommend chromosomal screening in azoospermic and severe oligozoospermic men, because they are assumed to have the highest risk of abnormalities. We performed a retrospective cohort study in azoospermic men and men eligible for ICSI. We determined the prevalence of chromosomal abnormalities in relation to sperm concentration and compared our data to studies in the literature. A high prevalence of chromosomal abnormalities in azoospermic men was found, but no difference in the prevalence of abnormalities was seen between different sperm concentration categories in non-azoospermic men. This raises the question of who should be screened for chromosomal abnormalities before ICSI treatment. Considering the costs and benefits, we would propose limiting screening to infertile couples with non-obstructive azoospermia.

Key words: chromosomal abnormality / ICSI / male infertility / sperm concentration / guidelines

Introduction

It is generally assumed that the prevalence of chromosomal abnormalities, numerical as well as structural aberrations, is increased in infertile men (first reported by [Chandley et al., 1975](#)), and that the risk of abnormalities is inversely related to sperm concentration ([Chandley, 1979](#); [De Braekeleer and Dao, 1991](#); [Yoshida et al., 1997](#); [Tuerlings et al., 1998](#); [Peschka et al., 1999](#); [Vincent et al., 2002](#)). No correlation between sperm motility or morphology and prevalence of chromosomal abnormalities has been found ([Matsuda et al., 1991](#); [Pandiyan and Jequier, 1996](#); [Van Assche et al., 1996](#); [Haidl et al., 2000](#); [Gekas et al., 2001](#); [Bonduelle et al., 2002](#)).

The reported prevalence of chromosomal abnormalities in infertile men varies among studies from 3 to 19% (summarized in [Martin, 2008](#)). This might be due to differences in the population studied, since in some studies all men attending fertility clinics were karyotyped, while in others only azoospermic or oligozoospermic men were tested. Furthermore, the definition of oligozoospermia varies widely, as some studies only include males with sperm counts <10 million/ml, while others report on men with sperm counts <30 million/ml.

After the introduction of ICSI as a means to enable men with poor sperm quality to father their own children, there have been concerns

of facilitating transmission of genetic abnormalities to their offspring. Therefore, guidelines have been issued about karyotyping men before starting ICSI. However, guidelines and the practice of chromosomal testing are not consistent and vary among countries.

The American Society of Reproductive Medicine (ASRM) recommends that karyotyping should be offered to men who have non-obstructive azoospermia or severe oligozoospermia (defined as <5–10 million sperm/ml) prior to performing ICSI with their sperm ([AUA and ASRM, 2006](#)). In the UK, the National Institute for Clinical Excellence (NICE) guideline states that men should be karyotyped if the indication for ICSI is a 'severe deficit of semen quality' or non-obstructive azoospermia. The definition of severe deficit of semen quality, however, is not given in the guideline ([NICE, 2004](#)). Karyotyping men with a total motile sperm count <1 million is recommended by the Dutch Society of Obstetrics and Gynaecology and, irrespective of sperm quality, karyotyping is considered a prerequisite for ICSI treatment ([NVOG, 1999](#)).

Because of inconsistent guidelines and ill-defined sperm limits for screening infertile men for chromosomal abnormalities, we performed a retrospective cohort study in men visiting our fertility clinic and looked for comparable studies in the literature. We determined the prevalence of chromosomal abnormalities in relation to sperm

concentration, in order to assess which group of men has the highest risk of abnormalities.

Materials and Methods

Cohort study

All male partners of couples applying for ICSI, irrespective of their sperm quality and all azoospermic men visiting the fertility clinic of the University Medical Centre Groningen between November 1994 and October 2007 were offered a chromosomal analysis in accordance with the prevailing guideline of the Dutch Society of Obstetrics and Gynaecology. Data were collected retrospectively by chart review, and only men whose results of chromosomal analysis and at least one sperm analysis were available, participated in the study. In the Netherlands, no ethical board approval is required for retrospective chart review and collection of anonymized data.

Men were defined as having a chromosomal abnormality in case of a numerical or structural gonosomal aberration, a translocation or inversion. Polymorphisms like the pericentric inversion in chromosome nine and heterochromatic variants were considered normal (Gardner and Sutherland, 2004). To allow comparison of prevalences of chromosomal abnormalities according to sperm quality, sperm concentration was categorized. Because no uniform categories of sperm concentration exist, we used cut-off levels frequently mentioned in the literature (azoospermia, 1, 5, 10 and 20 million/ml).

Literature

We searched the literature for studies reporting on prevalence of chromosomal abnormalities in male partners of couples eligible for ICSI, in which sperm concentration was given in relation to the abnormalities found. We only included studies presenting data in accordance with the sperm concentration categories we used in our cohort study. We excluded studies in which the karyotypes found were not explicitly given, and studies in which less than 20 metaphases were analysed. Twenty metaphases is commonly considered the minimum criterion to exclude clinically relevant mosaics (Wiktor et al., 2009). Studies presenting known polymorphisms as chromosomal abnormalities were also excluded. Furthermore, studies reporting on chromosomal analysis of spermatozoa were not taken into account.

Results

In our cohort study, 1222 men were included, of whom 38 (3.1%) had a chromosomal abnormality. In the literature eight studies were identified that met our inclusion criteria. These studies represent a population of 3807 men, and in 295 cases (7.7%) a chromosomal abnormality was reported. The prevalences of chromosomal abnormalities according to the different categories of sperm concentration for the studies reported in the literature and for our cohort are given in Table I.

The confidence intervals (CIs) show that our results are comparable with the results reported in the literature in all sperm concentration categories. When all data were considered, no significant differences were found between the percentages of chromosomal abnormalities in the different sperm concentration categories, except for azoospermia. Significantly more chromosomal abnormalities were found in azoospermic men (15.4%) than in non-azoospermic men (2.5%).

Discussion

It is assumed that males with poor sperm concentration carry a high risk of chromosomal abnormalities, as is reflected by the recommendations in the various guidelines on karyotyping infertile men. In our cohort and in the literature, however, the prevalence of chromosomal abnormalities did not differ significantly between the different sperm concentration categories, apart from a significantly higher prevalence in the group of azoospermic men.

In men with normal sperm concentration [i.e. >20 million/ml (WHO, 1999)], prevalences of chromosomal abnormalities of 2.3% in our cohort and 3.1% in the literature were found. This questions the assumption that sperm concentration is the single best parameter for identifying a population at risk for chromosomal abnormalities as indicted by the recommendations in the ASRM and NICE guidelines. All men included in our cohort and in the literature reviewed were eligible for ICSI. Oligozoospermia was not the only indication for ICSI, as ICSI was offered to couples in case of normal sperm concentration but poor sperm motility or morphology, or in case of fertilization failure in a previous IVF treatment in spite of apparently normal sperm quality. An association between sperm motility or sperm morphology and chromosomal abnormalities in men has not been reported in the literature (Matsuda et al., 1991; Pandiyan and Jequier, 1996; Van Assche et al., 1996; Haidl et al., 2000; Gekas et al., 2001; Bonduelle et al., 2002). However, not all these studies exclusively investigated men eligible for ICSI—most focused on a broader population of infertile men or even men with normozoospermia. It may be suggested that when ICSI is indicated despite normal sperm concentration, men carry a higher risk of having a chromosomal abnormality. Furthermore, fertilization failure in IVF may reflect poor oocyte quality, but it may also be considered as an indicator of poor fertilizing ability of the sperm because of an aberrant chromosomal make-up and a chromosomal abnormality in the male.

In our cohort and in the literature, the highest prevalence of chromosomal abnormalities was found in men with azoospermia. In our retrospective cohort study, it was not possible to distinguish between males with obstructive azoospermia and men with non-obstructive azoospermia. The study of Vincent et al. was the only study meeting the inclusion criteria of our review in which a distinction was made between non-obstructive and obstructive azoospermia. They reported a prevalence of chromosomal abnormalities of 16.7 and 2.1%, respectively (Vincent et al., 2002). This indicates that non-obstructive azoospermic males carry a higher risk of chromosomal abnormalities.

In all non-azoospermic males tested in our cohort and in the literature, the percentage of abnormal karyotypes was 2.5% (95% CI 2.0–3.0%). Very few studies on karyotyping have been performed in large, non-selected groups, and therefore reference data are rare. In unselected newborns the prevalence of chromosomal abnormalities was 0.8% (CI 0.8–0.9%) (Nielsen and Wohler, 1991), and in normozoospermic sperm donors of proven fertility an abnormal karyotype was noted in 0.4% (CI 0.3–0.5%) of cases (Ravel et al., 2006).

There are several reports on the prevalence of abnormal karyotypes in infertile populations. Chromosomal abnormalities were found in 1.0% (CI 0.5–1.4%) of normozoospermic male partners of infertile couples visiting fertility clinics (Clementini et al., 2005). In female partners of infertile couples, irrespective of the cause of

Table 1 Prevalence of chromosomal abnormalities in azoospermic men and men eligible for ICSI.

Sperm concentration (million/ml)	Males investigated (N)	Abnormal karyotypes (N)	Abnormal karyotypes (%)	95% confidence interval (%)	Reference
Azoospermia ^a	86	15	17.4	9.4–25.5	Akgul et al. (2009)
	50	8	16.0	5.8–26.2	Martinez-Garza et al. (2008)
	108	21	19.4	11.9–26.9	Mohammed et al. (2007)
	77	9	11.7	4.5–18.9	Cruger et al. (2003)
	49	3	6.1	0–12.8	Bor et al. (2002)
	358	67	18.7	14.7–22.8	Gekas et al. (2001)
	792	111	14.0	11.6–16.4	Vincent et al. (2002)
Total literature	1520	234	15.4	13.6–17.2	
	79	12	15.2	7.1–23.3	Present study
All data	1599	246	15.4	13.6–17.2	
>0–≤1	47	1	2.1	0–6.2	Cruger et al. (2003)
	149	3	2.0	0–4.3	Bor et al. (2002)
	24	2	8.3	0–19.3	van der Ven et al. (1997)
Total literature	220	6	2.7	0.6–4.9	
	319	10	3.1	1.2–5.0	Present study
All data	539	16	3.0	1.5–4.4	
>1–≤5	92	3	3.3	0–7.0	Cruger et al. (2003)
	94	3	3.1	0–6.6	Bor et al. (2002)
	39	1	2.6	0–7.6	van der Ven et al. (1997)
Total literature	225	7	3.1	0.8–5.4	
	250	3	1.2	0–2.6	Present study
All data	475	10	2.1	0.8–3.4	
>5–≤10	628	27	4.3	2.7–5.9	Vincent et al. (2002)
	40	1	2.5	0–7.3	van der Ven et al. (1997)
Total literature	668	28	4.2	2.7–5.7	
	211	3	1.4	0–3.0	Present study
All data	879	31	3.5	2.3–4.7	
>10–≤20	583	3	0.5	0–1.1	Vincent et al. (2002)
	34	0	0		van der Ven et al. (1997)
Total literature	617	3	0.5	0–1.0	
	191	6	3.1	0.6–5.6	Present study
All data	808	9	1.1	0.4–1.8	
>20	90	1	1.1	0–3.3	Cruger et al. (2003)
	27	2	7.4	0–17.3	Bor et al. (2002)
	430	13	3.0	1.4–4.6	Gekas et al. (2001)
	10	1	10.0	0–28.6	van der Ven et al. (1997)
Total literature	557	17	3.1	1.6–4.5	
	172	4	2.3	0.1–4.5	Present study
All data	729	21	2.9	1.7–4.1	

^aCategory not further specified.

infertility, abnormal karyotypes were noted in 3.2% (95% CI 2.9–3.6%) (Scholtes et al., 1998; van der Ven et al., 1998; Peschka et al., 1999; Haidl et al., 2000; Gekas et al., 2001; Morel et al., 2004; Clementini et al., 2005; Kaye et al., 2006; Riccaboni et al., 2008). When considering only female partners of ICSI couples, in 4.1% (CI 3.4–4.9%) a chromosomal abnormality was found (Gekas et al., 2001; Morel et al., 2004; Riccaboni et al., 2008). This is an unexpected high prevalence, because in ICSI couples the male factor predominates as the cause of infertility and female partners are usually considered as representatives of the normal population. The risk of chromosomal abnormalities might be increased in both male and female partners of infertile couples when compared with newborns and sperm donors.

The guidelines of the ASRM, NICE and Dutch Society of Obstetrics and Gynaecology on genetic testing in infertile males are based on assumed high prevalences of chromosomal abnormalities in azoospermic and severely oligozoospermic men. We confirmed a significantly increased prevalence of chromosomal abnormalities in azoospermic males (15.2%), justifying screening these men for chromosomal abnormalities.

However, we did not find a significant difference between the prevalence of abnormal karyotype in men with (severe) oligozoospermia or normal sperm concentration but requiring ICSI. This prevalence of 2.5% in non-azoospermic infertile men in our study questions whether this whole group should be chromosomally screened. Karyotyping and genetic counselling are relatively costly and costs and benefits of testing should be weighed. Karyotyping may identify the cause of male infertility, giving the couple some relief. However, this will be the case in only a small minority of infertile couples. If the main objective of karyotyping is to identify couples at risk of viable offspring with unbalanced structural chromosomal abnormalities, one should note that the incidence of balanced rearrangements in oligozoospermic men is ~1% (O'Flynn O'Brien et al., 2010). For most balanced rearrangements, the risk of a child with congenital anomalies due to a chromosomal unbalance has been shown to be very small (Franssen et al., 2006). In oligozoospermic men, the a priori risk of a child with an unbalanced karyotype due to a paternal balanced rearrangement is less than 1 in 10 000. This low risk can be considered acceptable in the light of the commonly applied threshold of 1 in 250 used in prenatal Down syndrome screening. Therefore, we would propose to limit screening for chromosomal abnormalities to infertile couples with non-obstructive azoospermia.

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